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M. Schroeder, D. Labudde

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Stabilizing Regions in Membrane Proteins

**Frank Dressel, Annalisa Marsico, Anne Tuukkanen,
Rainer Winnenburg, Michael Schroeder, and Dirk Labudde**

Biotechnologisches Zentrum , TU Dresden,
Tatzberg 47/49, 01307 Dresden, Germany
E-mail: firstname.lastname@biotec.tu-dresden.de

Around one third of a typical genome consists of membrane proteins. Misfolding of membrane proteins can often be linked to diseases, so that it is of great importance to understand, which residues and interactions are crucial for its stability. We developed a coarse-grained model to predict stabilizing regions in membrane proteins. We compare the model to experimental data from Single Molecule Force Spectroscopy (SMFS) and literature to evaluate the effects of mutations on function and stability of five membrane proteins. The aim of this study is to describe all these data in an unified context, the interaction energies of amino acids in a coarse grained model to gain a deeper insight into membrane proteins.

1 Introduction

Integral membrane proteins account for about 20%-30% of the open reading frames in a typical genome but, despite their central importance for all organisms, the number of known structures remains small giving raise to the need for sequence based methods.

Single Molecule Force Spectroscopy (SMFS) allows detecting and locating interactions stabilizing a membrane protein. During continuous stretching of the molecule, the applied forces and the extension are measured. The resultant force-distance curve reflects subsequent unfolding events¹, the so called force peaks. The so-called Worm-like-chain model (WLC)² relates the position of these unfolding barriers to positions in the primary sequence of the protein. Besides the SMFS data, there are a lot of mutation experiments in the literature, where the effect of a certain mutation on stability and function of a membrane protein is discussed.

In this work, we developed a coarse grained model, which is able to describe the above mentioned experimental results. To the best of our knowledge, this is the first time, that such a simplified model is used for predicting correctly unfolding barriers and estimating the influence of mutations based on sequence.

2 Methods

For the representation of a protein, we constrained ourself to the C_α/C_β atoms of each amino acid. To estimate the energy of the interaction between amino acids, we defined a solvation energy e_i based on the probability distribution for each amino acid in an membrane protein to be inside (solved) or outside of the protein³. Inside means facing other amino acids, outside means facing the phospholipids. The combination of two solvation energies results in a contact energy for an interaction between two spatial neighbored amino acids.

Based on the tertiary structure we estimate the energy E_i with which each amino acid i interacts with the rest of the protein by summing up the interaction energies between i and all other amino acids. Two amino acids interact if they are not direct neighbors in sequence and if their C_β atoms are less than 8\AA apart. This gives an energy profile containing the energy E_i for each amino acid in the protein. A deep valley in this profile gives rise to an energy barrier in the model.

To estimate the influence of a mutation based on the sequence only, we calculate a so called solvation energy profile, which contains for each amino acid i the solvation energy e_i . This profile is, in contrast to the energy profile, independent from the tertiary structure of a protein.

3 Results

For our analysis, we used the following five membrane proteins: Bacteriorhodopsin, Halorhodopsin, Bovine rhodopsin, Na^+/H^+ antiporter and Aquaporin-1. Up to now, these are the only proteins, for which SMFS data are available.

3.1 Prediction of Unfolding Barriers

For the five proteins mentioned above we predicted the barriers based on their energy profiles. For the comparison with the experiment, we considered the published barrier position from the experiment plus an experimental error of $\Delta_{exp} = \pm 4$ amino acids. This is due to systematic errors in the experiment like intrinsic movement of the cantilever. These errors are reported to be in the range of 3-7 amino acids⁴, depending on the used cantilever.

On average 61% of the barriers are detected by our method. A match between a predicted barrier and an experimentally determined one is established if their boundaries are within a certain distance.

A remarkable result is the following: all unfolding barriers for all proteins that, according to SMFS measurements, are found having 100% of probability of occurrence (main peaks) are correctly predicted by our method. This suggests that major unfolding barriers in the experiments are due to energetic reasons. Minor events are more difficult to detect with this approach.

Energy profiles can also be used for detecting smaller stabilizing regions in a protein. The helix C in bacteriorhodopsin for example is known for its important part in the proton pumping pathway⁵. The mutations T90A and T90V in helix C for example decrease the activity down to 10% and 20% compared to the wild type⁶. This is thought to be partly due to the missing stabilizing interaction of the threonine. Interestingly, alanine and valine are both more repulsive in our model than threonine itself. These mutations have a destabilizing effect on this region in helix C.

3.2 Predict Effects of Mutations Based on the Sequence

To quantify the ability of the model to predict the influence of mutations based on sequence alone, we screened the literature for single point mutations, which influence stability or function of the used membrane proteins. For this, we applied a text mining approach,

which searches in PubMed abstracts. We only used the publications, where a single point mutation was discussed in the context of stability or stability related function. If an appropriate mutation was found in the literature, we compared the solvation energies of both residues to decide, if the mutation was stabilizing, slightly stabilizing, slightly destabilizing and destabilizing. 25 out of 35 mutational effects reported in the literature correlate with the predictions from the energy profiles and thus can be related to stability issues in terms of amino acid interactions.

4 Summary

We used a coarse grained model to assess the influence of interactions between amino acids in membrane proteins. It is based on the probability distribution for an amino acid to be inside or outside of an membrane protein. We found that major SMFS unfolding barriers are related to strongly interacting amino acids, whereas minor barriers are related to non-energetic reasons. The activity of bacteriorhodopsin and the effect of mutations at site Thr90 is related to an increase of the interaction energy of this amino acid if mutated to Ala or Val. Although these two use cases require knowledge about the tertiary structure of a protein, it is possible to extend the scope of the model to a purely sequence based method. Compared with a list compiled from literature, 25 out of 35 mutations can be related to energetic reasons in the model by this sequence-only approach. Concluding, the proposed coarse grained interaction scheme can be applied successfully to a wide range of applications in the context of membrane proteins.

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